

FORM PTO-1390
(REV 10-95)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. §371**

MERCK 2251

U.S. APPLICATION NO. (If known, see 37 CFR §1.5)

09/868098

INTERNATIONAL APPLICATION NO.

INTERNATIONAL FILING DATE

PCT/EP99/09842

11 DECEMBER 1999

PRIORITY DATE CLAIMED

19 DECEMBER 1998

TITLE OF INVENTION

 $\alpha_v\beta_6$ INTEGRIN INHIBITORS

APPLICANT(S) FOR DO/EO/US


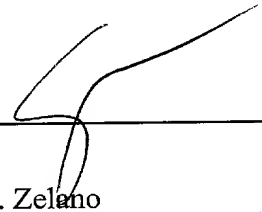
DIEFENBACH, Beate, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. §371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. §371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
 - ☒ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 CFR §1.55) 097868098		INTERNATIONAL APPLICATION NO. PCT/EP99/09842		ATTORNEY'S DOCKET NUMBER MERCK 2251	
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR §1.492 (a) (1) - (5)): Search Report has been prepared by the EPO or JPO..... \$860.00 International preliminary examination fee paid to USPTO (37 CFR §1.482)..... \$690.00 No international preliminary examination fee paid to USPTO (37 CFR §1.482) but international search fee paid to USPTO (37 CFR §1.445(a)(2))..... \$710.00 Neither international preliminary examination fee (37 CFR §1.482) nor international search fee (37 CFR §1.445(a)(2)) paid to USPTO..... \$1000.00 International preliminary examination fee paid to USPTO (37 CFR §1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 C.F.R. §1.492(e)). <input type="checkbox"/> 20 <input type="checkbox"/> 30					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	16 - 20 =	0	x \$ 18.00	\$0.00	
Independent claims	1 - 3 =	0	x \$ 80.00	\$0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$ 270.00		
TOTAL OF ABOVE CALCULATIONS =				\$860.00	
Reduction of 1/2 for filing by small entity, if applicable. A Verified Small Entity Statement must also be filed (Note 37 C.F.R. §§1.9, 1.27, 1.28).					
SUBTOTAL =				\$860.00	
Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 C.F.R. §1.492(f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30					
TOTAL NATIONAL FEE =				\$860.00	
Fee for recording the enclosed assignment (37 C.F.R. §1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§3.28, 3.31). \$40.00 per property.					
TOTAL FEES ENCLOSED =				\$860.00	
				Amount to be refunded:	
				charged:	
a. <input checked="" type="checkbox"/> A check in the amount of <u>\$860.00</u> to cover the above fees is enclosed.					
b. <input type="checkbox"/> Please charge my Deposit Account No. <u>13-3402</u> in the amount of \$_____ to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>13-3402</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 C.F.R. §§1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. §1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Customer Number 23,599 PATENT TRADEMARK OFFICE					
 23599			SIGNATURE  _____ Anthony J. Zelano NAME		
Filed: 14 JUNE 2001 AJZ:kmo			27,969 REGISTRATION NUMBER		

IN THE UNITED STATES DESIGNATED/ELECTED OFFICE

International Application No. : PCT/EP99/09842
International Filing Date : 11 DECEMBER 1999
Priority Date(s) Claimed : 19 DECEMBER 1998
Applicant(s) (DO/EO/US) : DIEFENBACH, Beate, et al.
Title: $\alpha_v\beta_6$ INTEGRIN INHIBITORS

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

SIR:

Prior to calculating the national fee, and prior to examination in the National Phase of the above-identified International application, please amend as follows:

IN THE CLAIMS:

7. (Amended) Peptide compounds of the formula I or II according to Claim 1 and their physiologically acceptable salts as medicaments.
10. (Amended) Pharmaceutical preparation comprising at least one medicament according to Claim 7 and, if appropriate, vehicles and/or excipients and, if appropriate, other active compounds.
11. (Amended) Use of peptide compounds according to Claim 1 and/or their physiologically acceptable salts for the production of a medicament for controlling disorders which are based on an expression and pathological function of $\alpha_v\beta_6$ integrin receptors.
13. (Amended) Recombinant DNA comprising a sequence which codes for a peptide section which corresponds to a peptide compound of Claim 1.

15. (Amended) Virus, characterized in that it possesses a coat protein which has a sequence which corresponds to a peptide compound of Claim 1.

REMARKS

The purpose of this Preliminary Amendment is to eliminate multiple dependent claims in order to avoid the additional fee. Applicants reserve the right to reintroduce claims to canceled combined subject matter.

Respectfully submitted,



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AJZ:jmm

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 7, 10-11, 13 and 15 have been amended as follows:

7. (Amended) Peptide compounds of the formula I or II according to Claims 1 to 6 and their physiologically acceptable salts as medicaments.

10. (Amended) Pharmaceutical preparation comprising at least one medicament according to ~~one of~~ Claims 7 to 9 and, if appropriate, vehicles and/or excipients and, if appropriate, other active compounds.

11. (Amended) Use of peptide compounds according to Claims 1 to 6 and/or their physiologically acceptable salts for the production of a medicament for controlling disorders which are based on an expression and pathological function of $\alpha_v\beta_6$ integrin receptors.

13. (Amended) Recombinant DNA comprising a sequence which codes for a peptide section which corresponds to a peptide compound of Claims 1-6.

15. (Amended) Virus, characterized in that it possesses a coat protein which has a sequence which corresponds to a peptide compound of Claims 1-6.

Inhibitors of the integrin $\alpha_v\beta_6$

The invention describes novel peptides which, as ligands of the integrin $\alpha_v\beta_6$, are biologically active.

5 These peptides all have a common structural motif, namely - **Asp Leu Xaa Xaa Leu** -, or in a preferred form - **Arg Xaa Asp Leu Xaa Xaa Leu Arg** -, where Xaa is any desired amino acid residue. The peptides according to the invention can be employed as effective inhibitors

10 of the $\alpha_v\beta_6$ integrin receptor and thus for the treatment of various diseases and pathological findings.

Integrins belong to the Class I family of heterodimers - transmembrane receptors which play an important part

15 in numerous cell-matrix and cell-cell adhesion processes (Tuckwell et al., 1996, Symp. Soc. Exp. Biol. 47). They can be roughly divided into three classes: the β_1 integrins, which are receptors for the extracellular matrix, the β_2 integrins, which are

20 activatable on leucocytes and are "triggered" during inflammatory processes, and the α_v integrins, which affect the cell response during wound healing and other pathological processes (Marshall and Hart, 1996, Semin. Cancer Biol. 7, 191).

25 The integrins $\alpha_5\beta_1$, $\alpha_{IIb}\beta_3$, $\alpha_8\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_6$ all bind to the Arg-Gly-Asp (RGD) peptide sequence, e.g. in the natural ligand fibronectin. Soluble RGD-containing peptides are able to inhibit the interaction of each of

30 these integrins with fibronectin. $\alpha_v\beta_6$ is a relatively rare integrin (Busk et al., 1992 J. Biol. Chem. 267(9), 5790), which is formed in increased amounts during repair processes in epithelial tissue and preferably binds the natural matrix molecules fibronectin and

35 tenascin (Wang et al., 1996, Am. J. Respir. Cell Mol. Biol. 15(5), 664). The physiological and pathological functions of $\alpha_v\beta_6$ are still not precisely known; however, it is suspected that this integrin plays an important part in physiological processes and disorders

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binding of fibronectin. This action can be demonstrated by the method which is described by J.W. Smith et al. in J. Biol. Chem. 265, 12267-12271 (1990).

5 The dependence of the origin of angiogenesis on the interaction between vascular integrins and extracellular matrix proteins is described by P.C. Brooks, R.A. Clark and D.A. Cheresh in Science 264, 569-71 (1994).

10 It was furthermore found that the new substances have very valuable pharmacological properties together with good tolerability and can be employed as medicaments. This is described in greater detail further below.

15 The peptide compounds according to the invention can furthermore be used *in vivo* as diagnostics for the detection and localization of pathological conditions in the epithelial system if they are equipped with the appropriate markers (e.g. the biotinyl radical)
20 according to the prior art. The invention also encompasses conjugates with other active compounds, such as cytotoxic active compounds, as well as conjugates with radiolabels for radiotherapy or PET diagnosis but also fusion proteins with marker proteins
25 such as GFP or antibodies, or therapeutic proteins such as IL-2.

The invention thus relates to peptide compounds of the formula I

30
$$W^1-X^1_n\text{Arg } X^2 \text{ Asp Leu } X^3X^4\text{Leu } X^5X^6_m-W^2 \quad \text{I}$$

in which:

$X^1, X^2, X^3, X^4, X^5, X^6$ each independently of one another are an amino acid residue, the amino acids
35 independently of one another being selected from a group consisting of Ala, Asn, Asp, Arg, Cys, Gln, Glu, Gly, Phe, His, Ile, Leu, Lys, Met, Nle, homo-Phe, Phg, Pro, Ser, Thr, Trp, Tyr or Val, and the amino acids mentioned possibly also being derivatized,

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W^2 is selected from a group OH, OR, NHR, NR_2 , NH_2 ,
 W^1 is H or an acyl radical

R is alkyl having 1-6 C atoms and

5

n, m each independently of one another are a number
from 0-15. In the cases in which m or n assumes a value
of greater than 1, the radicals X^1 and X^6 can each
independently of one another be identical or different.

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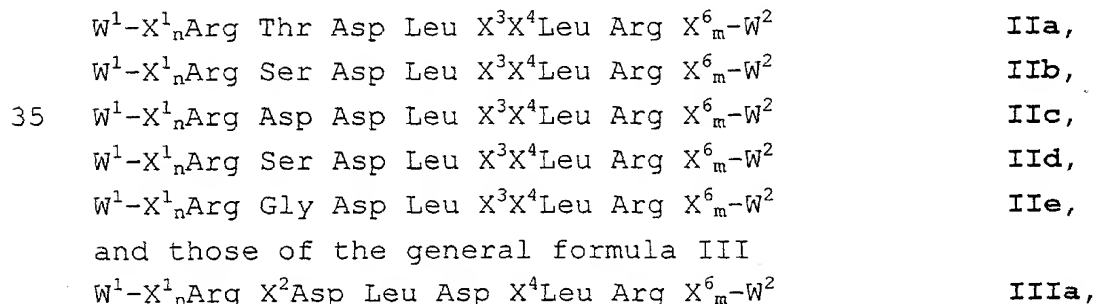
According to the invention, those amino acids or amino
acid residues are also encompassed which, starting from
the natural amino acids, are derivatized, or are
homologues or isomers thereof. The amino acid residues
15 are customarily linked to one another via their α -amino
and α -carboxyl groups (peptide bonding).

20

The invention furthermore preferably relates to those
peptide compounds in which X^2 is an amino acid residue
which was selected from the group consisting of Thr,
Ser, Asp and glycine, furthermore those peptide
compounds in which X^3 is an amino acid residue which was
selected from the group consisting of Asp, Glu, Arg,
Lys, His and Tyr, and finally those peptide compounds
25 in which X^4 is an amino acid residue which was selected
from the group consisting of Ser, Tyr, Thr, Gly and
Val.

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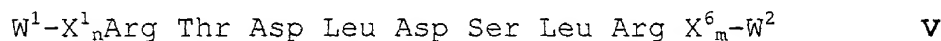
The preferred compounds (for meanings or abbreviations
see above and below) thus include those of the general
formula II



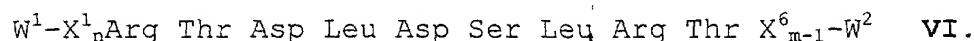
- $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } \text{Glu } X^4\text{Leu } \text{Arg } X^6_m-W^2$ IIIb,
 $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } \text{Arg } X^4\text{Leu } \text{Arg } X^6_m-W^2$ IIIc,
 $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } \text{Lys } X^4\text{Leu } \text{Arg } X^6_m-W^2$ IIId,
 $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } \text{His } X^4\text{Leu } \text{Arg } X^6_m-W^2$ IIIe,
5 $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } \text{Tyr } X^4\text{Leu } \text{Arg } X^6_m-W^2$ IIIIf,
 and those of the general formula IV

- $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } X^3\text{Ser } \text{Leu } \text{Arg } X^6_m-W^2$ IVa,
 $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } X^3\text{Tyr } \text{Leu } \text{Arg } X^6_m-W^2$ IVb,
 10 $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } X^3\text{Thr } \text{Leu } \text{Arg } X^6_m-W^2$ IVc,
 $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } X^3\text{Gly } \text{Leu } \text{Arg } X^6_m-W^2$ IVd,
 $W^1-X^1_n\text{Arg } X^2\text{Asp } \text{Leu } X^3\text{Val } \text{Leu } \text{Arg } X^6_m-W^2$ IVe.

15 Particularly preferred peptide compounds according to
 the invention are those of the formula V



20 and in this context in particular those of the formula
 VI



25 Finally, the following individual compounds are
 particularly preferred, those also being included which
 are modified at the N and C termini:

- (a) H-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-Tyr-Thr-Leu-
 OH
 30 (b) H-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-OH
 (c) Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-OH
 (d) Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-NH₂
 (e) H-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-OH
 (f) H-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-NH₂
 35 (g) H-Arg-Thr-Asp-Leu-Tyr-Tyr-Leu-Arg-Thr-Tyr-OH
 (h) Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-NH₂

The abbreviations mentioned above and below stand for
 the radicals of the following amino acids:

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25 If the abovementioned amino acids can occur in a number of enantiomeric forms, all these forms and also their mixtures are included above and below, e.g. as a constituent of the compounds of the formulae I-VI. Furthermore, the amino acids for example, as a
30 constituent of compounds of the formulae I-VI, can be provided with appropriate protective groups known per se.

The compounds of the formulae I-VI can have one or more chiral centres and therefore occur in various stereoisomeric forms. The formulae indicated include all these forms, in particular the D and L forms, especially in enantiomeric and racemic mixtures. Finally, the formulae I and II mentioned above and below according to the invention also include the

corresponding salts, in particular the corresponding physiologically acceptable salts.

So-called prodrug derivatives are also included in the compounds according to the invention, i.e. compounds of the formula I modified with, for example, alkyl or acyl groups, sugars or oligopeptides, which are rapidly cleaved in the body to give the active compounds according to the invention. Furthermore, derivatives are also included in the compounds according to the invention which consist of the actual peptides according to the invention and known marker compounds which make it possible to detect the peptides easily. Examples of such derivatives are biotinylated or fluorescence-labelled peptides.

In general, the peptides according to the invention are linear, but they can also be cyclized. The invention comprises not only the peptides of the formulae I to VI mentioned but also mixtures and preparations which in addition to these compounds according to the invention also contain other pharmacological active compounds or adjuvants which can influence the primary pharmacological action of the peptides according to the invention in a desired manner.

The compounds according to the invention and also the starting substances for their preparation are otherwise prepared by methods which are known per se and frequently employed, such as are described in the literature (e.g. in the standard works such as Houben-Weyl, Methoden der organischen Chemie [Methods of Organic Chemistry], Georg-Thieme-Verlag, Stuttgart), namely under reaction conditions which are known and suitable for the reactions mentioned. Use can also be made here of variants which are known per se.

Preferably, the peptides according to the invention can be prepared by means of solid-phase synthesis and subsequent removal and purification, as has been

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- described, for example, by Jonczyk and Meienhofer (Peptides, Proc. 8th Am. Pept. Symp., Eds. V. Hruby and D.H. Rich, Pierce Comp. III, p. 73-77, 1983, or Angew. Chem. 104, 1992, 375), or according to Merrifield (J. Am. Chem. Soc. 94, 1972, 3102). Otherwise, they can be prepared by customary methods of amino acid and peptide synthesis, such as are known, for example, from Novabiochem - 1999 Catalog & Peptide Synthesis Handbook of Calbiochem-Novabiochem GmbH, D-65796 Bad Soden, from numerous standard works and published patent applications. Biotinylated or fluorescence-labelled peptides/proteins can likewise be prepared by standard methods (e.g. E.A. Bayer and M. Wilchek in Methods of Biochemical Analysis, Vol. 26, The Use of the Avidin-Biotin Complex as a Tool in Molecular Biology; and Handbook of Fluorescent Probes and Research Chemicals, 6th Edition, 1996, by R.P. Haugland, Molecular Probes, Inc.; or alternatively WO 97/14716).
- Of course, the peptides of the formulae I-VI can also be liberated by solvolysis, in particular hydrolysis, or by hydrogenolysis of their 'functional derivatives. Preferred starting substances for the solvolysis or hydrogenolysis are those which, instead of one or more free amino and/or hydroxyl groups, contain corresponding protected amino and/or hydroxyl groups, preferably those which, instead of an H atom which is connected to an N atom, carry an amino protective group or which, instead of the H atom of a hydroxyl group, carry a hydroxyl protective group.
- The same applies to carboxylic acids which can be protected by substitution of their -CO-OH hydroxyl function by means of a protective group, e.g. as an ester.
- The expression "amino protective group" is generally known and relates to groups which are suitable for protecting (for blocking) an amino group from chemical reactions, but which are easily removable after the

desired chemical reaction has been carried out at other positions in the molecule. The expression "hydroxyl protective group" is likewise generally known and relates to groups which are suitable for protecting a hydroxyl group from chemical reactions, but which are easily removable after the desired chemical reaction has been carried out at other positions in the molecule. The liberation of the compounds from their functional derivatives is carried out - depending on the protective group used - e.g. using strong acids, expediently using TFA or perchloric acid, but also using other strong inorganic acids such as hydrochloric acid or sulfuric acid, strong organic carboxylic acids such as trichloroacetic acid or sulfonic acids such as benzene- or p-toluenesulfonic acid. Hydrogenolytically removable protective groups (e.g. CBZ or benzyl) can be removed by treating with hydrogen in the presence of a catalyst (e.g. of a noble metal catalyst such as palladium, expediently on a support such as carbon). The procedures are generally known and are not to be described in greater detail here.

As already mentioned, the peptides according to the invention include their physiologically acceptable salts, which can likewise be prepared by standard methods. Thus, a base of the formula I can be converted into the associated acid addition salt using an acid, for example by reaction of equivalent amounts of the base and of the acid in an inert solvent such as ethanol and subsequent evaporation. For this reaction, suitable acids are in particular those which yield physiologically acceptable salts. Thus inorganic acids can be used, e.g. sulfuric acid, nitric acid, hydrohalic acids such as hydrochloric acid or hydrobromic acid, phosphoric acids such as orthophosphoric acid, sulfamic acid, furthermore organic acids, in particular aliphatic, alicyclic, or araliphatic, aromatic or heterocyclic mono- or polybasic carboxylic, sulfonic or sulfuric acids, e.g.

formic acid, acetic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, lactic acid, tartaric acid, malic acid, citric acid, gluconic acid, ascorbic acids, nicotinic acid, isonicotinic acid, methane- or ethanesulfonic acid, ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, naphthalenemono- and disulfonic acids, laurylsulfuric acid. Salts with physiologically unacceptable acids, e.g picrates, can be used for the isolation and/or purification of the compounds according to the invention. On the other hand, an acid of the formula I can be converted into one of its physiologically acceptable metal or ammonium salts by reaction with a base. Possible salts in this case are in particular the sodium, potassium, magnesium, calcium and ammonium salts, furthermore substituted ammonium salts, e.g. the dimethyl-, diethyl- or diisopropylammonium salts, monoethanol-, diethanol- or diisopropylammonium salts, cyclohexyl- or dicyclohexylammonium salts, dibenzylethylenediammonium salts, furthermore, for example, salts with arginine or lysine.

The peptide compounds according to the invention can be employed, as already mentioned, as pharmaceutical active compounds in human and veterinary medicine, in particular for the prophylaxis and/or therapy of disorders in which epithelial cells are involved. Particularly to be emphasized in this context are disorders or inflammations or wound healing processes of the skin, the respiratory organs and the stomach and intestinal area, thus, for example, apoplexy, angina pectoris, oncoses, osteolytic illnesses such as osteoporosis, pathologically angiogenic illnesses such as, for example, inflammations, pulmonary fibrosis, ophthalmological illnesses, diabetic retinopathy, macular degeneration, myopia, ocular histoplasmosis, rheumatoid arthritis, osteoarthritis, rubeotic

glaucoma, ulcerative colitis, Crohn's disease, atherosclerosis, psoriasis, restenosis after angioplasty, in acute kidney failure or nephritis.

5 The invention accordingly relates to peptide compounds of the formulae defined above and below and in the claims including their physiologically acceptable salts as medicaments, diagnostics or reagents.

10 The invention relates in particular to appropriate medicaments as inhibitors for the control of disorders which are based indirectly or directly on expression of the $\alpha_v\beta_6$ integrin receptor, thus in particular on pathologically angiogenic disorders, thromboses,
15 cardiac infarct, coronary heart disorders, arteriosclerosis, tumours, osteoporosis, inflammations, infections and for influencing wound healing processes.

The invention also relates to appropriate
20 pharmaceutical preparations which comprise at least one medicament of the formulae I to VI and, if appropriate, vehicles and/or excipients.

The invention furthermore relates to the use of the
25 peptide compounds and/or their physiologically acceptable salts according to the claims and the description for the production of a medicament for controlling disorders which are based indirectly or directly on expression of the $\alpha_v\beta_6$ integrin receptor,
30 thus in particular in pathologically angiogenic disorders, thromboses, cardiac infarct, coronary heart disorders, arteriosclerosis, tumours, osteoporosis, inflammations, infections and for influencing wound healing processes. The medicaments according to the
35 invention or pharmaceutical preparations comprising them can be used in human or veterinary medicine. Possible excipients are organic or inorganic substances which are suitable for enteral (e.g. oral) or parenteral administration or topical application or for

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administration in the form of an inhalation spray and do not react with the new compounds, for example water, vegetable oils, benzyl alcohols, alkylene glycols, polyethylene glycols, glycerol triacetate, gelatin, carbohydrates such as lactose or starch, magnesium stearate, talc, petroleum jelly. Tablets, pills, coated tablets, capsules, powders, granules, syrups, juices or drops, in particular, are used for oral administration, suppositories are used for rectal administration, solutions, preferably oily or aqueous solutions, furthermore suspensions, emulsions or implants, are used for parenteral administration, and ointments, creams or powders are used for topical application. The new compounds can also be lyophilized and the lyophilizates obtained used, for example, for the production of injection preparations. The preparations indicated can be sterilized and/or can contain vehicles such as lubricants, preservatives, stabilizers and/or wetting agents, emulsifiers, salts for affecting the osmotic pressure, buffer substances, colourants, flavourings and/or one or more further active compounds, e.g. one or more vitamins.

For administration as an inhalation spray, sprays can be used which contain the active compound either dissolved or suspended in a propellant or propellant mixture (e.g. CO₂ or chlorofluorohydrocarbons). Expediently, the active compound is used here in micronized form, it being possible for one or more additional physiologically tolerable solvents to be present, e.g. ethanol. Inhalation solutions can be administered with the aid of customary inhalers.

The substances according to the invention can as a rule be administered in analogy to other known, commercially available peptides (e.g. described in US-A-4 472 305), preferably in doses between approximately 0.05 and 500 mg, in particular between 0.5 and 100 mg, per dose unit. The daily dose is preferably between approximately 0.01 and 20 mg/kg of body weight. The

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type, host cells which express the integrin $\alpha_v\beta_6$ can preferably be attacked (targeting).

Suitable viruses are, for example, adenovirus species
5 which have been used a number of times already as
vectors for foreign genes in mammalian cells. A number
of properties make them good candidates for gene
therapy, as can be inferred from S.J. Watkins et al.
Gene Therapy 4, 1004-1012 (1997) (see also J.
10 Engelhardt et al. Hum. Gene Ther. 4, 759-769 (1993)).
As can be found in A. Fasbender et al. J. Clin. Invest.
102, 184-193 (1998), the limited efficiency of the gene
transfer is a common problem in gene therapy by viral
and non-viral vectors. Using the above-described
15 additional ligand sequence for $\alpha_v\beta_6$ integrin in the coat
protein of the adenoviruses, an improvement in the
transfer, for example, of cystic fibrosis transmembrane
conductance regulator (CFTR) cDNA can be achieved.

20 Similarly to the work of T. Tanaka et al. Cancer
Research 58, 3362-3369 (1998), instead of the DNA for
angiostatin the DNA for the sequences of this invention
can also be used for cell transfections by means of
retroviral or adenoviral vectors.

25 The peptides according to the invention can also be
employed for use in gene therapy in man within a
liposome complex of lipid/peptide/DNA prepared for
transfection of cell cultures together with a liposome
30 complex consisting of lipid/DNA (without peptide). The
preparation of a liposome complex of lipid/DNA/peptide
is described, for example, in Hart S.L., et al 1998:
Lipid-Mediated Enhancement of Transfection by a Non-
Viral Integrin-Targeting Vector, Human Gene Therapy 9,
35 575-585.

A liposome complex of lipid/peptide/DNA can be
prepared, for example, from the following stock
solutions: 1 $\mu\text{g}/\mu\text{l}$ of lipofectin (equimolar mixture of

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DOTMA (= N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-tri-
methyammonium chloride) and DOPE (dioleylphosphatidyl-
ethanolamine), 10 µg/ml of plasmid DNA and 100 µg/ml of
peptide. For this, both DNA and peptide are dissolved
5 in cell culture medium. The liposome complex is
prepared by mixing the three components in a specific
weight ratio (lipid:DNA:peptide, for example,
0.75:1:4). Liposome DNA complexes for gene therapy in
man have already been described (Caplen N.J., et al
10 1995: Liposome-mediated CFTR gene transfer to the nasal
epithelium of patients with cystic fibrosis, Nature
Medicine 1, 39-46).

The invention thus also relates to the use of
15 appropriately modified recombinant DNA of gene-
releasing systems, in particular virus DNA, for the
control of illnesses which are based indirectly or
directly on an expression of $\alpha_v\beta_6$ integrin receptors,
thus in particular in pathologically angiogenic
20 disorders, thromboses, cardiac infarcts, coronary heart
disorders, arteriosclerosis, tumours, osteoporosis,
inflammations, infections and for influencing wound
healing processes.

25 The new compounds according to the invention can also
be used as integrin ligands for the preparation of
columns for affinity chromatography for preparing
integrins in pure form. The complex of an avidin-
derivatized support material, e.g. Sepharose, and the
30 new compounds of the formula I is formed by methods
known per se (e.g. E.A. Bayer and M. Wilchek in Methods
of Biochemical Analysis, Vol. 26, The Use of the
Avidin-Biotin Complex as a Tool in Molecular Biology).
Suitable polymeric support materials in this case are
35 the polymeric solid phases known per se in peptide
chemistry and having preferably hydrophilic properties,
for example crosslinked polysugars such as cellulose,
Sepharose or Sephadex®, acrylamide, polymers based on
polyethylene glycol or Tentakel polymers®.

Example 1

Preparation and purification of peptides according to the invention:

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In principle, the preparation and purification was carried out by means of Fmoc strategy with protection of acid-labile side chains on acid-labile resins using a commercially obtainable continuous flow peptide synthesizer according to the details of Haubner et al. (J. Am. Chem. Soc. 118, 1996, 17703).

10

In the following, the synthesis and purification is described by way of example for the peptide amide Ac-RTDLD₂SLR-NH₂. For the synthesis of peptide acids, an o-chlorotrityl chloride resin (Novabiochem) was coated with the appropriate C-terminal Fmoc amino acid according to the manufacturer's instructions and used in the synthesis apparatus according to the manufacturer's instructions (Milligen). The principal steps are washing - Fmoc protective group removal - washing - coupling with the next Fmoc amino acid - capping (acetylation) - washing. If an N-terminal acylation is desired after the last amino acid coupling, this is carried out after removal of the last Fmoc protective group using the appropriate activated acyl radical, e.g. the acetic anhydride.

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2 g of 9-Fmoc-aminoxanthenyloxy resin (Novabiochem, 0.37 mmol/g) were subjected to a coupling step, for 60 min in each case, in succession with 0.45 g each of hydroxybenzotriazole hydrate (HOBt), 0.5 ml of ethyldiisopropylamine, 4 equivalents each of diisopropylcarbodiimide (DIC) and Fmoc-amino acid in dimethylformamide (DMF), in a commercial synthesis apparatus and a typical procedure (apparatus and Milligen 9050 PepSynthesizerTM Handbook, 1987). Washing steps were carried out in DMF for 10 min, removal steps in piperidine/DMF (1:4 vol) for 5 min, N-terminal acetylations (capping) were carried out for 15 min using acetic anhydride/pyridine/DMF (2:3:15 vol). The

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amino acids Fmoc-Arg (Pmc), then Fmoc-Leu, then Fmoc-Ser(But), then Fmoc-Asp(OBut), then Fmoc-Leu, then Fmoc-Asp(OBut), then Fmoc-Thr(But), and finally Fmoc-Arg(Pmc) were used. After washing with DMF and isopropanol and subsequent drying in vacuo, 3.48 g of the N-terminally acetylated peptidyl resin, Ac-Arg(Pmc)-Thr(But)-Asp(OBut)-Leu-Asp(OBut)-Ser(But)-Leu-Arg(Pmc)-aminoxanthenyloxy resin, were obtained.

By treatment of this peptidyl resin with trifluoroacetic acid/anisole/dichloromethane (74 ml/3.7 ml/74 ml) for 4 h at room temperature, filtration, concentration in vacuo and trituration with diethyl ether, it was possible to obtain a precipitate of 0.6 g of peptide, Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-NH₂. Purification of the product was carried out by RP-HPLC on Lichrosorb RP18 (250-25, 7 µm, Merck KGaA) in 0.3% TFA using a gradient of 4% on 24% 2-propanol in 2 h at 8 ml/min and assessment by means of a UV flow-through photometer at 215 nm.

The product-containing fractions were freeze-dried. According to FAB-MS (Fast Atom Bombardment Mass Spectroscopy), the product obtained corresponded to the expectations: C₄₁H₇₃N₁₅O₁₅M 1015.5 g/mol; (M+H)⁺ is 1016. In the analytical HPLC on SuperSpher RP18e (250-4, Merck KGaA) in a gradient of 0-99% A (0.08 M phosphate pH 3.5, 15% acetonitrile) to B (0.03 M phosphate pH 3.5, 70% acetonitrile) in 50 min, at 1 ml/min, and detection at 215 nm, the purified product Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-NH₂ has a retention time of 7.22 min.

Further HPLC analyses were carried out in the two following systems:

System A: 0.3% trifluoroacetic acid having a gradient of 0-80% 2-propanol in 50 min on LichroSpher 60 RP-Select B[®] (250-4) (Merck KGaA, Darmstadt, Germany), at 1 ml/min, and detection at 215 nm.

System B: 0.1% trifluoroacetic acid having a gradient of 30-70% acetonitrile in 50 min on SuperSpher 100 RP18e® (250-4) (Merck KGaA, Darmstadt, Germany), at 1 ml/min and detection at 215 nm.

5

Example 2

The following peptides shown in Table 1 were prepared and purified analogously to Example 1.

10 Table 1:

Structure	MW (g/mol)	FAB-MS [M+H] found	Rt (HPLC) /min (System A)	Rt (HPLC) /min (System A)
RTDLDSLRTYTL	1453.6	1456	21.9	
DSLRTYTL	968.1	969	18.6	
RTDLDSL	818.9	820	18.6	23.6
DLDSLRTY	982.1	983	16.6	
RTDLDSLRL	975.1	975	13.5	
RTDLDSLRTY	1239.3	1239	16.6	
Ac-RTDLDSLRT	1118.2	1119	16.2	15.6
RTDLDSLRT	1076.2	1076	13.9	
RTDLPSLRTY	1221.4	1221	19.2	
RTDLDLRT-NH ₂	988.1	989	13.4	
Ac-RTDLDLRT-NH ₂	1030.2	1031	15.3	
RTDLYYLMDL	1302.5	1302		28.2
RTDLDSLRT-NH ₂	1075.2	1076	11.1	13.8
RTDLPLRTY	1249.4	1250	16.3	
RTDLYYLRTY	1363.5	1363		11.5
Ac-RTDLDSLRT-NH ₂	1117.2	1118	13.2	15.0
Ac-RTDLDSLRL-NH ₂	1015.5	1016	See Example 1	
TDLDSLRT	920.0	920		14.8
PVDLYYLMDL	1241.5	1241	36.1	

The comparison compounds used were known RGD peptides such as GRGDSPK, cyclo-(RGDFV), and the linear peptide DLYYLMDL.

Example 3

Preparation of an $\alpha_v\beta_6$ integrin preparation:

$\alpha_v\beta_6$ was obtained and purified in soluble transmembrane truncated form (Weinacker et al. 1994, J. Biol. Chem. 269, 6940) from a Baculovirus expression system according to recombination techniques known for $\alpha_v\beta_3$ (Mehta et al., 1998, Biochem. J. 330, 861) using 14D9.F8 antibody affinity chromatography (Mitjans et al., 1995, J Cell Sci. 108, 2825). Human α_v and β_6 cDNA clones are generally known and commonly accessible. The transfer vector pAcUW31 (Clontech Lab. Inc., USA), which allows simultaneous expression of two different target cDNAs, was employed in order to express transmembrane truncated $\alpha_v\beta_6$ from recombinant Baculovirus cells. To this end, an α_v transfer vector was prepared and transmembrane truncated $(\Delta TM)\alpha_v$ was excised from the plasmid $\alpha_v\Delta TM$ (pBac9) using the restriction enzymes EcoRI and XbaI (Mehta et al., for reference see above) and cloned into the BamHI cleavage site of pAcUW31 downstream of the polyhedrin promoter by means of blunt-end ligation. Transmembrane truncated β_6 cDNA was excised from the plasmid pCDNAneo β_6 (Weinacker et al., for reference see above) using the restriction enzymes EcoRI and XbaI and likewise cloned into the BamHI cleavage site of pAcUW31 downstream of the polyhedrin promoter by means of blunt-end ligation. The tandem vectors containing truncated α_v and β_6 were used in order to obtain recombinant Baculovirus (Mehta et al., for reference see above). The recombinant Baculoviruses were employed in order to infect High Five insect cells. The soluble receptor was obtained after culturing for 48-71 hours by passing the supernatant from the cell culture through affinity columns of the type indicated above and eluting at pH 3.1. All process steps were carried out at room temperature and in the absence of any detergents. The peak fractions were neutralized, concentrated and dialysed at 40°C and finally stored at -80°C. The

recombinant soluble human receptor thus obtained is biologically active and retains its ligand specificity. A similar preparation method used for soluble $\alpha_v\beta_3$ was described in EP 0846 702.

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Example 4:

$\alpha_v\beta_6$ /Fibronectin receptor binding test:

10 The prepared peptides according to the invention were bonded to the immobilized $\alpha_v\beta_6$ receptor in solution together with competitively acting fibronectin and the Q value was determined as a measure of the selectivity of the binding of the peptide to be tested to $\alpha_v\beta_6$. The Q value is in this case calculated from the quotient of
15 the IC_{50} values of test peptide and a standard. The standard used was the linear hepta-RGD peptide GRGDSPK (ref./Patent cf. Pytela et al. Science 231, 1559, (1986)).

In detail, the binding test was carried out as follows:

20

The immobilization of soluble $\alpha_v\beta_6$ receptor on microtitre plates was carried out by dilution of the protein solution in TBS++ and subsequent incubation overnight at 4°C (100 µl/well). Non-specific binding
25 sites were blocked by incubation (2 h, 37°C) with 3% (w/v) BSA in TBS++ (200 µl/well). Excess BSA was removed by washing three times with TBSA++. Peptides were serially diluted (1:10) in TBSA++ and incubated with the immobilized integrin (50 µl of peptide + 50 µl
30 of ligand per well; 2 h; 37°C) together with biotinylated fibronectin (2 µg/ml). Unbound fibronectin and peptides were removed by washing three times with TBSA++. The detection of the bound fibronectin was carried out by incubation (1 h; 37°C) with an alkaline phosphatase-coupled anti-biotin antibody (Biorad)
35 (1:20,000 in TBSA++; 100 µl/well). After washing three times with TBSA++, the colorimetric detection was carried out by incubation (10-15 min; 25°C, in the dark) with substrate solution (5 mg of nitrophenyl

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phosphate, 1 ml of ethanolamine, 4 ml of H₂O; 100 µl/well). The enzyme reaction was stopped by addition of 0.4 M NaOH (100 µl/well). The colour intensity was determined at 405 nm in an ELISA measuring apparatus and made equal to the zero value. Wells which were not coated with receptor were used as a zero value. The standard employed was GRGDSPK. The IC₅₀ values for the tested peptides were read off from a graph and the Q value of the peptide according to the invention was determined from this together with the IC₅₀ value of the standard peptide. The results of the test described are summarized in the following table:

Table 2

Structure	Q value = IC ₅₀ test peptide/ IC ₅₀ standard peptide
GRGDSPK	1.0 (IC ₅₀ = 400 nM)
cyclo-(RGDfV)	0.6
DLYYLMDL	Inactive (IC ₅₀ >50 µM)
RTDLDSLRTYTL	0.27
DSLRTYTL	Inactive (IC ₅₀ >50 µM)
RRDLDSL	2.5
DLDSLRTY	Inactive (IC ₅₀ >50 µM)
RTDLDSLRT	0.17
RTDLDSLRTY	0.10
Ac-RTDLDSLRT	0.029
RTDLDSLRT	0.11
RTDLDLRT-NH ₂	1.1
Ac-RTDLDLRT-NH ₂	0.5
RTDLYYLMDL	0.33
RTDLDSLRT-NH ₂	0.056
RTDLDEPLRTY	0.50
RTDLYYLRTY	0.042
Ac-RTDLDSLRT-NH ₂	0.013
TDLDSLRT	66
PVDLYYLMDL	Inactive (IC ₅₀ >50 µM)

Q values of less than 1 mean that they exhibit a relatively better binding to the receptor than, comparatively, the standard peptide, which seen in absolute terms already has a good binding in competition with the natural ligand fibronectin.

Example 5

Analogously to the preceding example, for comparison purposes integrin ligand binding tests were carried out with different integrins (e.g. $\alpha_v\beta_3$, $\alpha_v\beta_5$) and their corresponding ligands (e.g. vitronectin, fibrinogen).

Example 6:

General preparation of a DNA-liposome complex and use for gene therapy:

Lipid and DNA are mixed in the weight ratio 5:1 (lipid:DNA) in Krebs-HEPES solution (140mM NaCl, 1mM $MgCl_2$, 2mM $CaCl_2$, 6mM KCl, 10mM HEPES, 10mM D-glucose; pH 9.0). The individual dose here is 30 μg of DNA/200 μl . 200 μl of this 'lipid-DNA complex are applied to the nasal epithelium using a pump atomizer. This is repeated 10 times at an interval of 15 min. The total dose of DNA is 300 μg .

The following examples relate to pharmaceutical preparations:

Example A: Inection vials

A solution of 100 g of an active compound of the formula I and 5 g of disodium hydrogenphosphate are adjusted to pH 6.5 in 3 l of double-distilled water using 2 N hydrochloric acid, sterile-filtered, filled into injection vials, lyophilized under sterile conditions and aseptically sealed. Each injection vial contains 5 mg of active compound.

Example B: Suppositories

A mixture of 20 g of an active compound of the formula I is fused with 100 g of soya lecithin and 1400 g of cocoa butter, poured into moulds and allowed to cool. Each suppository contains 20 mg of active compound.

Example C: Solution

A solution of 1 g of an active compound of the formula I, 9.38 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 28.48 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 0.1 g of benzalkonium chloride in 940 ml of double-distilled water is prepared. It is adjusted to pH 6.8, made up to 1 l and sterilized by irradiation. This solution can be used in the form of eye drops.

Example D: Ointment

500 mg of an active compound of the formula I are mixed with 99.5 g of petroleum jelly under aseptic conditions.

Example E: Tablets

A mixture of 1 kg of active compound of the formula I, 4 kg of lactose, 1.2 kg of potato starch, 0.2 kg of talc and 0.1 kg of magnesium stearate is compressed to give tablets in a customary manner such that each tablet contains 10 mg of active compound.

Example F: Coated tablets

Analogously to Example E, tablets are pressed and are then coated in a customary manner with a coating of sucrose, potato starch, tragacanth and colourant.

Example G: Capsules

2 kg of active compound of the formula I are filled into hard-gelatin capsules in a customary manner such that each capsule contains 20 mg of the active compound.

Example H: Ampoules

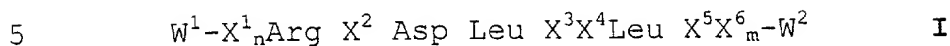
10 A solution of 1 kg of active compound of the formula I in 60 l of double-distilled water is sterile-filtered, filled into ampoules, lyophilized under sterile conditions and aseptically sealed. Each ampoule contains 10 mg of active compound.

Example I: Inhalation spray

14 g of active compound of the formula I are dissolved in 10 l of isotonic NaCl solution and the solution is
20 filled into customary spray containers having a pump mechanism. The solution can be sprayed into the mouth or nose. One puff of spray (approximately 0.1 ml) corresponds to a dose of approximately 0.14 mg.

Patent Claims

1. Peptide compounds of the formula I



in which:

10 $X^1, X^2, X^3, X^4, X^5, X^6$ each independently of one another are an amino acid residue, the amino acids independently of one another being selected from a group consisting of Ala, Asn, Asp, Arg, Cys, Gln, Glu, Gly, Phe, His, Ile, Leu, Lys, Met, Nle, homo-Phe, Phg, Pro, Ser, Thr, Trp, Tyr or Val, and the amino acids mentioned possibly also being

15 derivatized,
 W^1 is H or Ac,
 W^2 is OH, OR, NHR, NR_2 , NH_2 ,
 R is alkyl having 1-6 C atoms and
 n, m each independently of one another are a

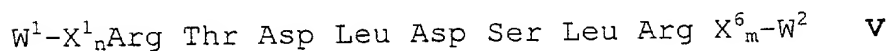
20 number from 0-15.

2. Peptide compounds according to Claim 1, in which X^2 is an amino acid residue which is selected from the group consisting of Thr, Ser, Asp or glycine.

- 25 3. Peptide compounds according to Claim 1, in which X^3 is an amino acid residue selected from the group consisting of Asp, Glu, Arg, Lys, His or Tyr.

- 30 4. Peptide compounds according to Claim 1, in which X^4 is an amino acid residue selected from the group consisting of Ser, Tyr, Thr, Gly or Val.

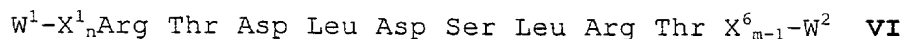
- 35 5. Peptide compounds according to Claim 1 as in the formula V



having the meanings indicated in Claim 1.

6. Peptide compound according to Claim 5 as in formula VI

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7. Peptide compounds of the formula I or II according to Claims 1 to 6 and their physiologically acceptable salts as medicaments.

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8. Medicament according to Claim 7 as an inhibitor for the control of disorders which are based on an expression and pathological function of $\alpha_v\beta_6$ integrin receptors.

15

9. Medicament according to Claim 8 for the control of thromboses, cardiac infarct, coronary heart disorders, arteriosclerosis, tumours, osteoporosis, fibrosis, inflammations, infections, psoriasis and for influencing wound healing processes.

20

10. Pharmaceutical preparation comprising at least one medicament according to one of Claims 7 to 9 and, if appropriate, vehicles and/or excipients and, if appropriate, other active compounds.

25

11. Use of peptide compounds according to Claims 1 to 6 and/or their physiologically acceptable salts for the production of a medicament for controlling disorders which are based on an expression and pathological function of $\alpha_v\beta_6$ integrin receptors.

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12. Use according to Claim 11 for the production of a medicament for controlling thromboses, cardiac infarct, coronary heart disorders, arteriosclerosis, tumours, osteoporosis, fibrosis,

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inflammations, infections, psoriasis and for influencing wound healing processes.

- 5 13. Recombinant DNA comprising a sequence which codes for a peptide section which corresponds to a peptide compound of Claims 1-6.
14. Recombinant virus DNA according to Claim 13.
- 10 15. Virus, characterized in that it possesses a coat protein which has a sequence which corresponds to a peptide compound of Claims 1-6.
- 15 16. Use of a virus according to Claim 15 for the production of a medicament for controlling disorders which are based on an expression and pathological function of $\alpha_v\beta_6$ integrin receptors.

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Abstract

The invention describes novel peptides which, as ligands of the integrin $\alpha_v\beta_6$, are biologically active.

5 These peptides all have a common structural motif, namely - **Asp Leu Xaa Xaa Leu** -, or in a preferred form -Arg Xaa **Asp Leu Xaa Xaa Leu** Arg - , where Xaa is any desired amino acid residue. The peptides according to

10 of the $\alpha_v\beta_6$ integrin receptor and thus for the treatment of various diseases and pathological findings.

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Docket No.
Merck 2251

Declaration and Power of Attorney For Patent Application English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

avB6 INTEGRIN INHIBITORS

the specification of which

(check one)

- ☐ is attached hereto.
☐ was filed on 11 December 1999 as United States Application No. or PCT International Application Number PCT/EP99/09842
 and was amended on _____
 (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

198 58 587.7 Germany 19 December 1999
 (Number) (Country) (Day/Month/Year Filed)



 (Number) (Country) (Day/Month/Year Filed)



 (Number) (Country) (Day/Month/Year Filed)



I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U.S.C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112. I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C.F.R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Fifth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of sixth inventor, if any	
Sixth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	